

# **Prevalence of subclinical mastitis and intramammary infections in camels with clinically healthy udders in Kenya**

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# Prevalence of subclinical mastitis and intramammary infections in camels with clinically healthy udders in Kenya

Förekomsten av subklinisk juverinflammation och intramammära infektioner hos kameler med kliniskt friska juver i Kenya

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## SUMMARY

For pastoralists in Kenya camel milk is an important source of nutrition and income. Previous studies have revealed that subclinical mastitis (SCM) is both common in camels and unknown amongst the pastoralists themselves. The purpose of this study was to investigate the prevalence of SCM and the prevalence of intramammary infections (IMI) in dairy camels (*Camelus dromedarius*) from two different ranch herds in the Laikipia district, Kenya, to examine teat morphology of these camels and to investigate if an association between teat morphology and SCM or IMI exists. In addition to this the association between teat morphology and three camel factors (age, number of parties and days in milk) will be examined.

A further aim was to investigate which bacterial species were present in IMI and the association between IMI and SCM. The possible association between IMI or SCM and three camel factors will be investigated.

All lactating camels with clinically healthy udders in the two herds were included in the study, 35 in total. For each camel milk samples were collected from each quarter and all quarters were examined by California Mastitis Test (CMT). One of the herds was visited twice and the other herd three times, and all lactating females were sampled on each occasion.

In total, 138 individual udder quarters from 35 camels were included in the study. Out of the 35 camels, 13 were sampled once, 13 were sampled twice, and 9 were sampled three times. One of the camels in the study had 2 blind teats and one sample was lost during transport to the lab. In total, this resulted in 261 quarter milk samples and 253 CMT-results. In addition, each udder was photographed from both sides with a ruler underneath for scale (35 udders altogether). Information about age, days in milk (DIM) and number of parities was collected for each camel.

Bacteriological analysis was done locally at Analabs laboratories Ltd, Nairobi, Kenya and species determination using MALDI-ToF at Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. In this study SCM was defined as CMT  $\geq 3$  and IMI as bacterial growth of monoculture. The teat width (TW) and teat length (TL) were measured digitally using the pictures of the udders. T-test and univariable mixed-effect linear regression analyses were used to investigate associations between TL or TW and age, parity, DIM, prevalence of SCM and IMI. The Chi<sup>2</sup> test was used to compare age, parity, DIM, IMI and SCM.

The quarter level prevalence of SCM was 11% and the animal level prevalence was 29%. The quarter level prevalence of IMI was 48% and the animal level prevalence was 89%. Average TL was 50mm and average TW was 32mm. There was a strong correlation between TL and TW. Front teats were significantly longer and wider than hind teats but no significant difference was found between left and right side for either front or hind teats. A significant association was found between TW and parity, where camels in parity one or two had wider teats than camels in parity four. No significant association between TL or TW and SCM or IMI was found. The bacterial species identified in quarter milk samples with IMI were: *S. simulans* (26%), *Str. pluranimalium* (17%), *S. hyicus* (5%), *S. haemolyticus* (5%), *S. chromogenes* (5%), *S. aureus* (5%), *S. agalactiae* (5%), *S. gallolyticus* (5%), *S. ovis* (2%), *C. efficiens* (2%) and *S. epidermidis* (2%). It was not possible to identify 21% of the bacterial species found. The association between IMI and SCM was not significant in this study.

## SAMMANFATTNING

Kamelmjölk är en viktig källa till näring och inkomst för pastoralister i Kenya. Tidigare studier har visat att subklinisk mastit (SCM) är både vanligt bland kamelerna och okänt hos pastoralisterna. Syftet med denna studie var att undersöka prevalensen av SCM samt prevalensen av intramammära infektioner (IMI) hos mjölkkamer (*Camelus dromedarius*) i två hjordar på två rancher i Laikipia distriktet, Kenya, att undersöka spenmorfologin hos dessa kameler samt att undersöka om det finns något samband mellan SCM eller IMI och spenmorfologi. Dessutom ska sambandet mellan spenmorfologi och tre kamelfaktorer (ålder, antal dräktigheter, laktationsstadie) undersökas.

Ytterligare ett mål var att undersöka vilka bakterier som hittas hos kameler med IMI, och om något samband mellan SCM och IMI kan ses. Även den möjliga associationen mellan SCM eller IMI och de tre kamelfaktorerna ska studeras.

Alla lakterande kameler med kliniskt friska juver i de två hjordarna inkluderade i studien, totalt 35 stycken. Alla fyra juverdelar undersöktes med California Mastitis Test (CMT) och ett mjölkprov samlades in ifrån varje juverdel. En av hjordarna besöktes två gånger, den andra tre gånger och alla lakterande kameler provtogs varje gång. Totalt resulterade detta i 261 mjölkprov och 253 CMT-prov. Utöver detta fotograferades alla 35 juver ifrån båda sidor, med en linjal under juvret för att få en skala i bilden. Information om ålder, laktationsstadium (DIM) samt antal laktationer samlades in för varje kamel.

Den bakteriologiska odlingen utfördes av Analabs laboratories ltd, Nairobi, Kenya och artbestämning med MALDI-ToF gjordes på Sveriges lantbruksuniversitet (SLU), Uppsala, Sverige. I denna studie så definierades SCM som ett CMT-värde under 3 och IMI som bakteriell växt i monokultur. Spenlängd (TL) och spenvidd (TW) mättes digitalt i bilderna på juvren. T-test och enkel linjär regressions analys användes för att undersöka om det fanns något samband mellan TL eller TW och ålder, antal laktationer, DIM, SCM eller IMI.

Prevalensen av SCM var 13% på juverfjärdedelsnivå och 29% på kamelnivå. Prevalensen av IMI var 48% på juverfjärdedelsnivå och 91% på kamelnivå. Medel för TL var 50mm och medel för TW var 32mm. En stark korrelation mellan TL och TW fanns. Framspenar var signifikant längre och vidare än bakspenar, men ingen signifikant sidoskillnad sågs. Det fanns ett signifikant samband mellan TW och antal laktationer, kameler i första eller andra laktationen hade vidare spenar än kameler i sin fjärde laktation. Ingen signifikant association sågs mellan TL eller TW och SCM eller IMI. De bakterier som identifierades mjölkprover med IMI var; *S. simulans* (26%), *Str. pluranimalium* (17%), *S. hyicus* (5%), *S. haemolyticus* (5%), *S. chromogenes* (5%), *S. aureus* (5%), *S. agalactiae* (5%), *S. gallolyticus* (5%), *S. ovis* (2%), *C. efficiens* (2%) och *S. epidermidis* (2%). Vi lyckades inte artbestämma 21% av bakterierna som hittades. Associationen mellan IMI och SCM var inte signifikant i denna studie.

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## **ABBREVIATIONS**

The following abbreviations are used in the present study.

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CM	Clinical mastitis
SCM	Subclinical Mastitis
CMT	California mastitis test
IMI	Intramammary infection
SCC	Somatic cell count
TL	Teat length
TW	Teat width

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## INTRODUCTION

According to FAO there are about 28 million camels in the world. Of the world's camel population, approximately 27 million are one-humped and about 1 million are two-humped. Generally, the two-humped camels can be found in Asia while the one-humped ones are found in the Middle East, North Africa and Australia. Almost 24 million of the world's camels live in Africa, while almost 3 million occur in the Middle East and Australia (FAO, 2015). The camels are generally kept for production of milk, meat and sometimes as working animals or for racing.

For the pastoralists in northeast Africa, milk from camels is an important source of both nutrition and income, especially during the dry periods. Pastoralists are defined as people who make their living by tending to a herd of livestock. Often this includes some degree of mobility, such as moving livestock to new pastures or to areas with more water. Pastoralists generally live in areas where traditional agriculture is limited due to the climate. In Africa this includes the drylands (Rota and Sperandini, 2009).

Since the milk production of the camels is important for the nomadic pastoralists of northeast Africa, udder inflammation (mastitis), as one of the most common diseases of dairy animals, is an important condition amongst milk-producing camels. Depending on the symptoms mastitis is usually classified as clinical or subclinical. Clinical mastitis (CM) causes visible changes in the milk, swelling of the udder and can cause fever and an altered general condition. Not all symptoms are present in all cases of CM. Sometimes CM are further classified into chronic or acute depending on the duration of the disease, and as mild, moderate or severe depending on the severity of the symptoms. The disease is known amongst the pastoralists and many traditional treatments of doubtful effect exist (Abbas and Omer, 2005). Equally important, but lesser known amongst the pastoralists, is subclinical mastitis (SCM) (Regassa et al., 2013). In SCM there are no clinical symptoms visible to the naked eye but the composition of the milk is altered, especially the milk somatic cell count (SCC), which consists mainly of white blood cells that increases in response to an infection. A SCM is almost always the result of an intramammary infection (IMI).

As SCM is not visible, diagnostic tools such as an optical cell counter (either manual or automatic) or the California mastitis test (CMT) are needed to detect affected udder quarters. The CMT is easy to use in the field but to what extent pastoralists with camels know about and use this test has not been investigated. However, the CMT has been evaluated as a tool for detecting camels with IMI (Younan et al., 2001). It is important to identify camels with SCM in order to prevent spread of bacteria within and between camels. Mastitis, both clinical and subclinical, causes a decrease in milk production (Kashongwe et al., 2017), causing a decline in food security and income for the pastoralists (Elhadi et al., 2015). It is also a health concern for the consumers, especially since some of the milk is sold and transported long distances without cooling and then consumed unpasteurized (Musinga et al., 2008). In addition, mastitis can potentially cause considerable pain for the affected animal.

An important risk factor for mastitis in camels is the lack of milking hygiene practices such as washing of hands before milking, washing of udders before and after milking, and milking

camels with healthy udders before milking camels with mastitis (Ahmad et al., 2011). In pastoralist camel herds such hygiene practices are very uncommon (Kashongwe et al., 2017). Other factors associated with an increased prevalence of mastitis in camels are increasing age, increased number of parities, presence of lesions on the udder, presence of tick infestations of the udder and the use of traditional teat tying to prevent the calf from drinking (Abera et al., 2010a; Regassa et al., 2013; Younan et al., 2001). In dairy cattle, longer and wider teats have been shown to be associated with an increased risk for mastitis (Guarín and Ruegg, 2016; Sharma et al., 2016). Since other predisposing factors for mastitis found for camels are similar to those in dairy cattle it can be suspected that the morphology of the camel teats might also predispose for mastitis. To our knowledge this association has not been investigated in camels.

Several studies have been performed investigating the presence of bacteria in udder quarters of camels, but many of these studies took place in other countries than Kenya (Ahmad et al., 2011; Al-Dughaym and Fadlelmula, 2015; Husein et al., 2013; Obied et al., 1996) where conditions may be different. However, to our knowledge, no study has investigated the prevalence of different species of coagulase negative staphylococci in milk from camels.

## **Purpose**

The purpose of this study was to investigate the prevalence of SCM and the prevalence of IMI in dairy camels from two ranch herds in the Laikipia district, Kenya, to examine teat morphology of these camels and to investigate if an association between teat morphology and SCM or IMI exists. In addition to this the association between teat morphology and three camel factors (age, number of parties and days in milk) will be examined.

A further aim was to investigate which bacterial species were present in IMI, and the association between IMI and SCM. The association between IMI or SCM and the camel factors will also be studied.

The study was carried out in two stationary camel herds in Laikipia, Kenya. The herds were chosen due to ease of access and willingness of the personnel to participate in the study. Camels showing signs of CM were excluded from the study.

## **LITERATURE REVIEW**

### **Production of camel milk in Kenya**

Agriculture and forestry are by far the most important economic sectors in Kenya, and almost 50% of the output in these sectors comes from livestock (FAO, 2015; Behnke and Muthami, 2011). Milk is the most economically important livestock product, with milk from camels making up almost 19% of all milk produced in Kenya. This is not surprising, considering that the country is home to approximately 2.9 million camels (*Camelus dromedarius*), about 10% of the world's population. The Kenyan camel population is the third largest in the world, dwarfed only by the Somalian and Sudanese ones (FAO, 2015). An overwhelming majority (>99%) of the camels are kept by pastoralists (Field, 2005). Camel milk is an important source of nutrition in pastoral households, especially during the dry season when it makes up about a third of the total food intake. Sales of camel milk are also a significant and increasingly important source of income during both the dry and wet seasons (Elhadi et al., 2015; Anderson et al., 2012). Most of the milk is sold and consumed unpasteurized; only 0.3% of the milk is pasteurized (Musinga et al., 2008).

### **The camel as dairy animal**

The camel is well adapted to a harsh, dry climate and is able to produce milk for a long period of time, whereas the dry climate limits the milk production of other dairy animals such as cattle or goats (Bekele et al., 2002; Degen, 2007). This ability is important in the Kenyan drylands where climate change makes unpredictable droughts more common. Recently, many pastoralist communities that have not traditionally kept camels, have diversified their livestock herds and included some camels in an attempt to mitigate the effect of climate change. The production of camel milk in Kenya increased by 300% in the last 10 years; the majority of the increase occurred in the years after the drought of 2005/2006 (FAO, 2015; Kagunyu and Wanjohi, 2014).

Overall, the composition of camel milk is similar to that of cow milk, especially when the animals are kept in the same kind of environment. Camel milk contains slightly less protein and lactose than cow milk but no significant difference in total energy content can be measured when both animals are kept under the same conditions (Soliman, 2005). An interesting difference can be seen, however, when comparing the vitamin content of the milk. Compared to milk from cows, milk from camels contains less vitamin A and B<sub>2</sub>, an equal amount of vitamin E and considerably more vitamin C. The increased vitamin C might be a nutritional advantage in the drylands where the availability of fruit and vegetables is low (Farah et al., 1992). There is also a significant variability in the milk composition depending on the breed of camel, the season and the environment it is kept in (Sawaya et al., 1984). In a dry environment, or in the case of drought, the milk yield of the camel does not decrease, as seen in other species, but the dry matter content decreases (Yagil and Etzion, 1980).

There are certain key differences between the camel and the cow that are important when discussing dairy performance. Compared to cattle, the camel has both a longer lifespan and a longer lactation. The average lactation length is 12 months, but this varies depending on management, feeding and in which season the calf is born. The camel has a slow reproductive cycle, the average age at first parturition is about 5 years and the average interval between calves is 2 years. On the other hand a camel usually produces 8-9 calves during its lifetime

(Abdussamad et al., 2011). In camels, the presence of the calf is very important both to initiate milk let-down and to maintain lactation. If the calf survives until weaning, which happens at approximately 12 months' age, the camel produces approximately 65% more milk during the lactation than if the calf dies. Unfortunately, calf mortality in pastoralist herds is high; 20-25% do not survive their first year (Kaufmann, 2005).

Similarly to the cow, the camel has an udder with four quarters and four teats. However, in the camel each quarter consists of two distinctly separate glands, each connected to a separate udder cistern and then in turn connected to separate teat cisterns. In contrast the cow has only one teat cistern, one udder cistern and one gland per quarter (Figure 1) (Abshenas et al., 2007; Kausar, 2001). The volume of both the cistern and teat canal is smaller in the camel compared to the cow and the volume of cisternal milk is lower compared to both cows and goats (Ayadi et al., 2009, 2013).

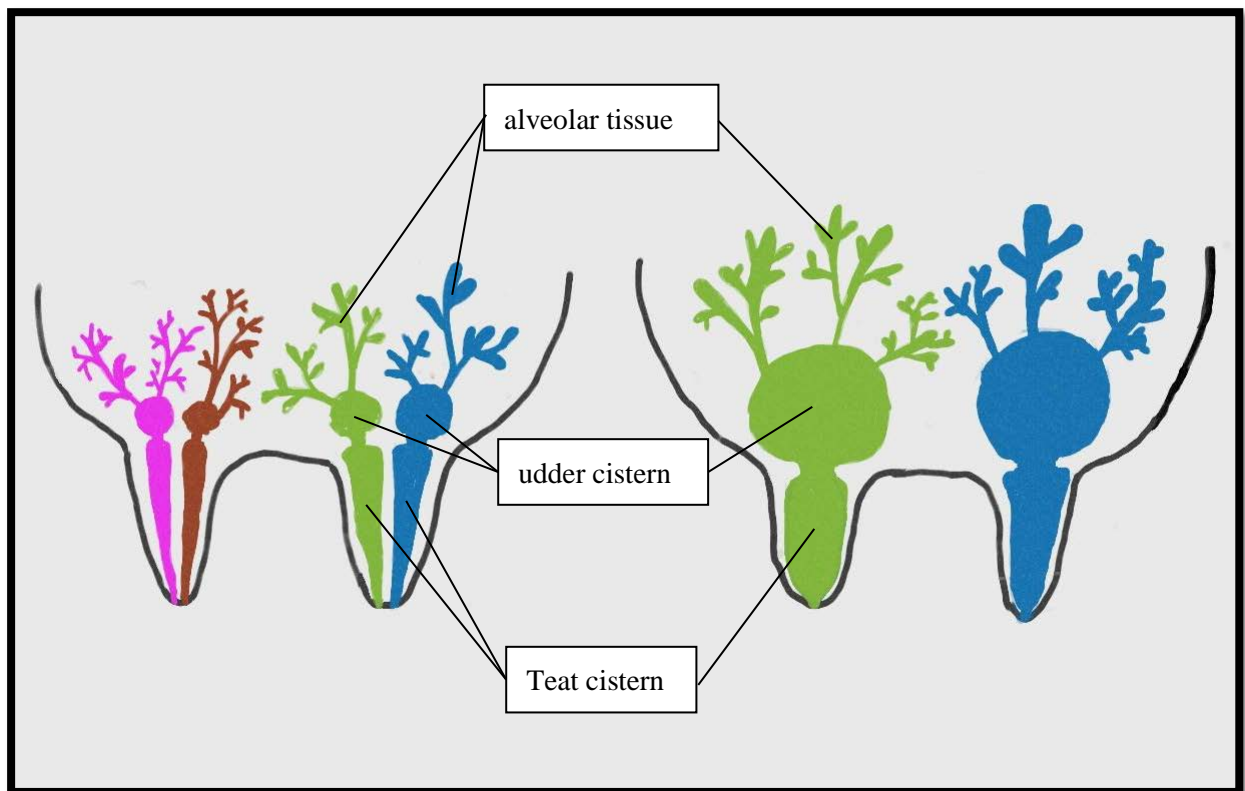


Figure 1. *Schematic overview of selected parts of the internal anatomy of the teat and udder in camels (left) and cattle (right) (Daniel Goncalves 2017).*

A camel produces between 2 and 10 liters of milk a day. The higher end of the range represents the production of a well-fed dairy camel kept under intense management and milked by machine (Musaad et al., 2013). A camel kept under pastoralist conditions produces about 4 liters of milk in **addition** to the amount consumed by her calf (Bekele et al., 2002).

It is possible to milk camels by machine and this is commonly practiced in countries where camels are kept under more intense management systems (Atigui et al., 2014), for example, in

the Emirates (Figure 2). In Kenya, however, all camels are milked by hand. When milking by hand, the skill of the milker is essential for maximizing the milk yield. As milk let-down is so short, an increased milking speed means a greater milk yield. Herdsmen that balance the collecting bucket on their knee and milk two teats at a time are likely to get a higher milk yield than herdsmen that hold the bucket in one hand and milk with the other. Some tribes milk both sides at the same time, but tribes that allow the calf to suck on one side and milk the other are likely to get a better milk let-down and a better milk yield (Simpkin, 1995).

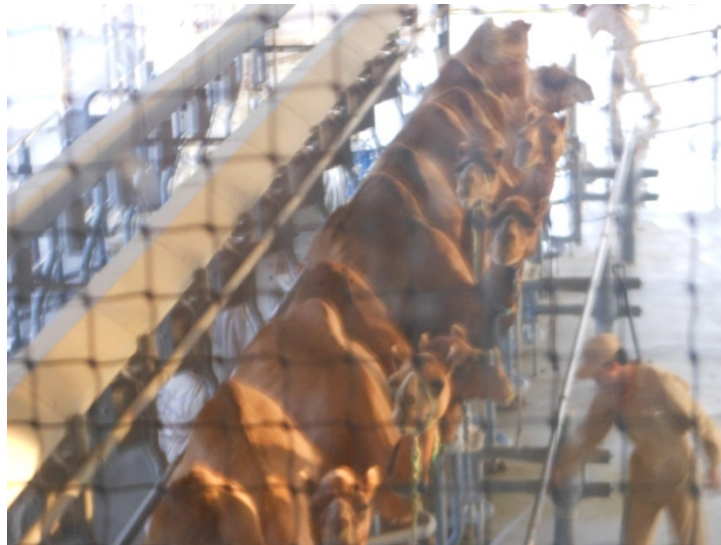


Figure 2. *Camels milked by machine at Camelicious in Dubai, United Arab Emirates. (JM Morrell 2015)*

In Kenya, almost all pastoralist camels are kept in a stationary enclosure called a *boma* (Figure 3) during the night. The enclosure is traditionally made of branches from thorny bushes but some ranches use modern materials such as chain-link fences. The calves are kept separate from their mothers during the night and are then released one at a time in the morning and allowed to suck to initiate milk let-down. In traditional/pastoralist camel keeping, the camels are milked twice in the morning with approximately one hour in between, and are then herded during the day to browse on any available vegetation, returning to the *boma* at sunset. The exact management varies between different pastoralists.





Figure 3. *Camels waiting to leave the boma after the morning milking at Loisaba Conservancy. (Daniel Goncalves 2016).*

## **Mastitis**

Mastitis is defined as an inflammation of the udder and can result from a number of different causes, such as physical trauma, thermal injury or IMI by microorganisms. The most common cause is infection by microorganisms, most commonly by different bacteria. Mastitis is often divided into two subcategories, CM and SCM.

The definition of CM is an inflammation of the udder where clinical signs can be observed without the use of any special equipment. Signs of CM can include local changes in the udder such as swollen, hard and sore udder, and abnormalities in the milk such as clotting, thickening and changes in color (P Iyer, 2014; Rebhun et al., 2008), as well as systemic signs such as fever and altered general condition. Not all symptoms are present in all cases of CM. Depending on the severity of the symptoms CM is usually classified as either mild, moderate or severe. Sometimes CM is further classified as chronic or acute depending on the duration of the disease.

In the case of SCM, the inflammation does not cause visible abnormalities in the udder or the milk, but the milk composition is altered and the number of inflammatory cells in the milk increases (de Graaf and Dwinger, 1996).

Symptoms of CM are known amongst the camel herding pastoralists, at least in Ethiopia (Regassa et al., 2013). However, SCM is a less-well known condition in the pastoral communities of northeast Africa (Regassa et al., 2013; Abera et al., 2010a).

### ***In-field Diagnostics of SCM***

Since SCM can't be detected during an ordinary clinical examination of the affected animals, other diagnostic methods must be used.

Somatic cells, mostly white blood cells, are always present in the milk, but during an IMI the SCC rises as a part of the body's response to infection. Other factors, such as parity, days in milk (DIM) and breed can affect the SCC, but IMI has a bigger impact on the increase in SCC than the other factors. In cows this phenomenon is well understood and the rise in SCC can be used as a means of detecting SCM. However, as yet for camels a precise SCC-threshold has not been established and defined as SCM and different authors use different SCC-values as the definition of SCM.

The CMT is an indirect way of measuring the SCC of the milk. It works by adding a reagent to the milk that lyses the somatic cells. This causes the milk-reagent mixture to thicken, and the amount of thickening can be scored visually according to a set of criteria (Table 1). In cattle, detection of SCM is routinely done with the CMT (Rebhun et al., 2008).

Since camels show the same kind of increase in SCC during infection as cattle, the CMT can be used in the same way in this species (Abdurahman et al., 1995). There is a positive correlation between CMT-score and IMI-status for camels with SCM, and the CMT has a reported sensitivity of 60 - 95% and specificity of 30% -95% for detection of IMI when using  $\geq 3$  as threshold (Abdurahman et al., 1995; Younan et al., 2001). A CMT score of either  $\geq 2$  or  $\geq 3$  is commonly used as the definition of SCM in camels in the literature. Somatic cell counts of  $>300\,000$  cells/ml have been suggested as a viable cut-off value for the detection of IMI (Niasari-Naslaji et al., 2016). Another study showed that an SCC of 415 000 was the average in camels with IMI (Guliye et al., 2002).

Table 1. *The CMT-scores, the scoring criteria for each score and the corresponding somatic cell counts (SCC) in cattle. The SCC-values for cattle are used since values for camels are not available (adapted from Schalm and Noorlander (1957))*

Scandinavian CMT – International Score	Criteria	SCC (cells/ml)
1	Negative No thickening or gel formation, fluid stays homogenous	0 – 200,000
2	Trace Mild thickening of fluid when vessel is tilted	200,000 – 400,000
3	1 Clear thickening of fluid when vessel is tilted	400,000 – 1200,000
4	2 Clear thickening of fluid with a tendency of gel formation that disappears when vessel is not rotated	1200,000 – 5000,000
5	3 Clear thickening and gel formation that remains when vessel is not rotated	$>5000,000$



### **Prevalence of camel mastitis**

The prevalence of mastitis in camels, both clinical and subclinical, is reported to be between 30% and 46% in lactating camels held under pastoralist conditions (Table 2). As for other milk producing animals, SCM in camels is more common than CM, with a ratio of 4-8:1 when looking at prevalence at the animal level (Ahmad et al., 2011; Husein et al., 2013; Regassa et al., 2013).

Table 2. *The prevalence of mastitis in different studies conducted on camels kept under pastoralist conditions.*

Reference	Number of animals	Country	Clinical mastitis -CL <sup>1</sup>	Subclinical mastitis -CL <sup>1</sup>	Mastitis total -CL <sup>1</sup>	Quarters affected	Definition of mastitis
Ahmad et al., 2011	150	Pakistan	8%	38%	46%	68%	SFMT <sup>2</sup> ≥2
Regassa et al., 2013	348	Ethiopia	5%	39%	44%	19%	CMT <sup>3</sup> ≥2
Husein et al., 2013	384	Ethiopia	5%	25%	30%	26%	CMT <sup>3</sup> ≥2
Seifu and Tafesse, 2010	161	Ethiopia	8%	67%	76%	74%	CMT <sup>3</sup> ≥3
Mohammed et al., 2005	137	Ethiopia	10%	-	-	48%	CMT <sup>3</sup> ≥3
Obied et al., 1996	763	Sudan	20%	27%		47%	CMT <sup>3</sup> ?

1. CL=Camel level 2. SFMT = Surf Field Mastitis Test 3. CMT=California mastitis test

### **Prevalence of IMI**

The prevalence of IMI in camels have been reported to be 44%-67%, for details see Table 3 (Abdurahman et al., 1995; Matofari et al., 2005; Obied et al., 1996).

Table 3. *The prevalence of IMI in different studies conducted on camels kept under pastoralist conditions*

Reference	Abdurahman et al., 1995	Obied et al., 1996	Matofari et al., 2005
No of quarter milk samples	391	757	435
County	Sudan	Sudan	Kenya
IMI prevalence	170 (44%)	347 (46%)	290 (67%)
Mixed growth prevalence	32 (8%)	31 (4%)	-
No growth	189 (48%)	379 (50%)	-

### **Bacteriological findings in IMI in cases of SCM**

Many different bacteria have been isolated in milk from camels with SCM. Most of the species found are Gram-positive, and *Staphylococcus* spp. and *Streptococcus* spp. are the most common (Al-Dughaym and Fadlilmula, 2015; Regassa et al., 2013). For an overview of bacteria isolated in milk from camels with SCM, see Table 4.

Table 4. *The species of bacteria found in quarter milk samples from camels with subclinical mastitis and their prevalences from different studies conducted on camels kept under pastoralist conditions*

Bacterial Species	Ahmad et al., 2011	Regassa et al., 2013	Seifu and Tafesse, 2010
No of quarter milk samples	n=57	n=304	n=174
<i>Staphylococcus aureus</i>	13 (25 %)	39 (13 %)	6 (3 %)
CNS	8 (15 %)	56 <sup>1</sup> (18 %)	57 (32 %)
<i>Staphylococcus hyicus</i>	-	27 (9 %)	-
<i>Streptococcus intermedius</i>	-	12 (4 %)	-
<i>Streptococcus dysgalactiae</i>	-	32 (11 %)	32 (18 %)
<i>Streptococcus uberis</i>	-	13 (4 %)	11 (6 %)
<i>Streptococcus agalactiae</i>	8 (15 %)	22 (7 %)	5 (3 %)
<i>Corynebacteriae</i> spp.	4 (8 %)	-	13 (7 %)
<i>Bacillus</i> spp.	6 (12 %)	23 (8 %)	11 (6 %)
<i>Escherica coli</i>	7 (13%)	21 (7 %)	9 (5%)
<i>Micrococcus</i> spp.	-	12 (4 %)	-
<i>Klebsiella</i> spp	-	5 (2 %)	-
<i>Enterobacter</i> spp.	-	15 (5 %)	-
<i>Candida</i> spp.	3 (6 %)	-	-
Yeast	3 (6 %)	-	-
Mixed growth <sup>2</sup>	-	26 (10%)	-
No growth	5 (10%)	40 (13%)	26 (10%)

<sup>1</sup> Including *S.hyicus* and *S.intermedius*. <sup>2</sup> ≥2 species

### **Coagulase negative staphylococci**

Coagulase negative staphylococci (CNS) belong to a heterogeneous group of staphylococci that was created historically in order to differentiate *Staphylococcus aureus* from the rest of the staphylococci (Becker et al., 2014). Traditionally CNS were thought to be of minor importance as udder pathogens but recently they have emerged as one of the most common causes of this disease in dairy cattle. However, the prevalence and importance of CNS differs between countries. The species in the CNS group usually cause mastitis with mild clinical symptoms or SCM; it is more common to identify CNS in SCM than in CM (Pyörälä and Taponen, 2009; Taponen and Pyörälä, 2009).

Commonly identified CNS species in milk from dairy cattle are *S. epidermidis*, *S. simulans*, *S. chromogenes*, *S. haemolyticus* and *S. xylosus* (Piessens et al., 2011; Pyorala and Taponen, 2009; Thorberg et al., 2009). There are, however, considerable differences in CNS-species identified

between different herds, which suggests that herd level factors influence the establishment of CNS in a herd (Piessens et al., 2011).

In camels, CNS is found in 8-57% of SCM cases (Table 4). Not much is known about the prevalence of individual CNS species, but *S. intermedius* has been isolated in milk from camels with SCM (Regassa et al., 2013).

### ***Staphylococcus aureus***

*Staphylococcus aureus* is considered to be one of the most important contagious udder pathogens that can cause different forms of CM and SCM. In Swedish dairy cows, IMI with *S. aureus* is found in 21% of CM cases and 19% of SCM cases (Ericsson Unnerstad et al., 2009; Persson et al., 2011). It is a common udder pathogen, and is hard to eliminate once the pathogen is established within a herd. *S. aureus* is not an obligate pathogen of the udder; it may also colonize the skin, vagina, tonsils and other areas of the body (Rebhun et al., 2008). The most common mode of transmission is from infected quarters to healthy quarters via the hands of milkers or contaminated milking equipment (Zadoks et al., 2002). Calves may acquire the infection by suckling on an infected quarter and then spread the infection by suckling on an uninfected quarter (Mitsuda et al., 1996; Rebhun et al., 2008).

*Staphylococcus aureus* has been isolated in milk from camels with SCM, the prevalence ranging from 6 to 39% (Ahmad et al., 2011; Regassa et al., 2013; Seifu and Tafesse, 2010). Infection with *S. aureus* is common in camel herds in Kenya; 11% of animals have been shown to be infected (Younan et al., 2001).

### ***Streptococcus agalactiae***

*Streptococcus agalactiae* is an udder-associated pathogen, transmitted from udder to udder when milking hygiene is neglected. It might also be transmitted to the calf via infected milk. *Streptococcus agalactiae* was historically considered to be a major source of SCM in dairy cattle but it can also cause CM (Keefe, 1997; Rebhun et al., 2008). Recent efforts at eradication/control-programs in herds have led to a decrease in prevalence (Zadoks and Fitzpatrick, 2009).

The bacteria has been isolated from the milk of camels with SCM, with a prevalence ranging from 8% to 22% (Regassa et al., 2013; Seifu and Tafesse, 2010), but also from skin infections, joints with septic arthritis, uterine infections and from secondary respiratory infections (Younan et al., 2000).

### ***Streptococcus uberis***

*Streptococcus uberis* is mostly considered as an environmental udder pathogen, and can cause both CM and SCM. It is common in bedding material, and infections are often associated with suboptimal cleaning of the udders prior to milking (Rebhun et al., 2008). Amongst dairy cows in Sweden, infection with *S. uberis* was found in 11% of CM cases and 8% of SCM cases (Ericsson Unnerstad et al., 2009; Persson et al., 2011).

The bacterium has been isolated in milk from camels with SCM, with quarter level prevalence ranging from 11% to 13% (Regassa et al., 2013; Seifu and Tafesse, 2010).

### ***Streptococcus dysgalactiae***

*Streptococcus dysgalactiae* is considered to be both a contagious and an environmental udder pathogen (Rebhun et al., 2008). Infection with *S. dysgalactiae* has been found in 16% of CM cases and 9% of SCM cases in Swedish dairy cows (Ericsson Unnerstad et al., 2009; Persson et al., 2011) .

The bacterium has been isolated in milk from camels with SCM, prevalence ranging from 11% to 13% (Regassa et al., 2013; Seifu and Tafesse, 2010).

### **Coliforms**

Mastitis caused by *Escherichia coli*, *Klebsiella* spp. or *Enterobacter* spp. is often referred to as coliform mastitis; they are mostly environmental pathogens. These species are Gram-negative. Management that allows a build-up of coliforms in the environment increases the risk of coliform mastitis (Rebhun et al., 2008).

In camels, the majority of the bacteria isolated from milk samples are gram-positive, but coliforms have been isolated from camels with SCM (Ahmad et al., 2011; Husein et al., 2013; Regassa et al., 2013).

### ***Escherichia coli***

Amongst dairy cows in Sweden, *Escherichia coli* was found in 16% of CM cases and 3% of SCM cases (Ericsson Unnerstad et al., 2009; Waller and Unnerstad, 2004).

In camels, *Escherichia coli* was found in 7 -21% of SCM quarters.

### ***Klebsiella* spp.**

Mastitis caused by *Klebsiella* spp. was found in 4% of CM cases and in slightly less than 1% of SCM cases amongst Swedish dairy cows (Ericsson Unnerstad et al., 2009).

In camels, *Klebsiella* spp have been isolated in milk from camels with SCM with a prevalence of 5% (Regassa et al., 2013).

### ***Enterobacter* spp.**

Out of the three coliforms, *Enterobacter* spp is the one that is least commonly identified in milk from cows with CM and SCM. In Swedish dairy cows, *Enterobacter* spp. accounts for less than 1% of CM and has been found in milk from cows with SCM (Lindhagen and Waller, 2006)

One study on camels found *Enterobacter* spp. in 5% of samples from camels with SCM (Regassa et al., 2013).

## **Predisposing factors for mastitis in camels**

Few studies have been done on factors predisposing for mastitis in the camel compared to dairy cattle but those published have shown associations with the same kind of risk factors as in dairy cattle.

### ***Age and days in milk***

Increasing age has been shown to be related to an increased prevalence of mastitis, both with CM and SCM in camels (Abera et al., 2010a; Ahmad et al., 2011). Abera et al. (2010) showed a linear relationship between age and occurrence of mastitis. However, Regassa et al. (2013) found no association between age of the camel and prevalence of mastitis. Considering the periods of lactation, the prevalence of mastitis is highest during the first month of lactation, and second highest during the last two months of lactation (Ahmad et al., 2011; Regassa et al., 2013).

### ***Teat morphology***

To our knowledge, there are no studies on the association between teat morphology and IMI or mastitis in camels. However, both teat length and width have been associated with an increased milk yield in camels (Atigui et al., 2016). In cattle, teat length, width and shape have all been shown to be associated with the prevalence of IMI (Klaas et al., 2006). Cattle with longer or wider teats have been shown to have an increased prevalence of SCM and CM (Guarín and Ruegg, 2016; Sharma et al., 2016; Singh et al., 2014). For dairy cows, this increased prevalence might be related to the fact that teats with a larger diameter have an increased risk for slippage of the liner during machine milking (Baxter et al., 1992). Slippage of the liner causes a rapid loss of vacuum which might allow pathogens to be transported from the teat opening into the cistern of the teat (Mein et al., 2004). This effect would obviously not be seen in camels milked by hand-and the author of the present study has not been able to find any data on teat morphology and risk of IMI or mastitis in animals milked by hand.

### ***Lesions on the udder and presence of ticks***

The presence of abscesses and lesions on the udder has been shown to be associated with an increased risk of mastitis in the camel (Abera et al., 2010a; Ahmad et al., 2011; Regassa et al., 2013). Infections that cause skin lesions, such as camel pox have been reported to increase the spread of IMI between animals (Younan et al., 2001).

There are conflicting reports in the literature regarding tick infestations: (Abera et al., 2010a)) report a strong association between tick infestation of the udder, and Husein et al. (2013) noted that a high proportion of the camels that had mastitis also had tick-infested udders. On the other hand, Regassa et al. (2013) found no significant association between tick infestation of the udder and mastitis.

### ***Teat tying***

Teat tying is the traditional practice of tying together two of the camel's teats or tying sharp sticks and plant fibers to the teats in order to discourage the calf from suckling during the day (Seifu and Tafesse, 2010). This causes trauma to the teat and predisposes the udder for mastitis (Regassa et al., 2013). Despite this predisposition, some studies have reported that the practice can be found in 98% of pastoralist camel herds (Husein et al., 2013; Regassa et al., 2013).

### **Milking hygiene**

Milking hygiene includes factors such as cleaning the udder before milking, cleaning hands before milking and disinfection of the udder after milking. In addition, segregating animals with mastitis from the rest of the herd and milking them last is considered part of milking hygiene. Poor milking hygiene has been associated with an increase in the prevalence of mastitis in camels (Ahmad et al., 2011). None of the milking hygiene practices mentioned above are used by Kenyan pastoralists (Kashongwe et al., 2017).

### **Treatment of mastitis in camels**

In camels, CM is recognized by pastoralists and commonly treated with either intramammary infusions of antibiotics, traditional treatments, or a combination of both. In East Africa commonly used antibiotics are tetracycline, gentamicin, chloramphenicol, penicillin G or kanamycin (Abdelgadir, 2016). The usage of antimicrobials for animals is not regulated or monitored in Kenya (Fischer et al., 2013). Different kinds of antibiotics are readily available in any pharmacy in any urban center without a prescription. Tetracycline is one of the more popular ones in the study area (Laikipia County, Kenya). One study in Kenya found that only 50% of the bacterial isolates were sensitive to tetracycline, which the author suggested was due to widespread use of injectable tetracycline (Younan et al., 2001). Amongst pastoralists, various traditional remedies are common, especially when the distance to urban centers increases and the availability of medical drugs and veterinary intervention decreases (Abbas, 1997; Mathias, 2007; Shibia et al., 2013). These traditional treatments include the burning of certain herbs on, or close to, the udder (moxibustion) and cauterization or branding with an hot iron (Seifu and Tafesse, 2010). These treatments are often ineffective and lead to chronic damage to the udder and considerable suffering for the animal (Abbas and Omer, 2005).

### **Consequences of mastitis in camels**

Mastitis, both SCM and CM, results in an increased number of bacteria in the milk and thus in a lower hygienic quality of the milk. This poses a health risk for the consumer, especially since the milk is often transported long distances in plastic containers without any sort of cooling and is consumed unpasteurized (Musinga et al., 2008; Noor et al., 2013). Poor hygienic milk quality is also a constraint for the growth of the urban luxury camel milk market, which means a lost opportunity for an increased income for the pastoralists (Musinga et al., 2008). In addition, both CM and SCM cause decreased milk production (Kashongwe et al., 2017) and thus a direct decrease in both income and food availability for the pastoralists (Anderson et al., 2012; Elhadi et al., 2015).

For the camel itself, CM means pain and decreased welfare, and an risk for chronic damage to the teats and udder causing non-functional udder quarters - “blind” teats (Husein et al., 2013; Regassa et al., 2013). There is an hypothesis that pathogens, specifically *S. aureus* can infect the calves feeding on mastitis milk and thus possibly transfer antibiotic resistance or cause mastitis later in life, at least among dairy cows (Ivemeyer et al., 2009; Mitsuda et al., 1996; Rebhun et al., 2008). However, some evidence suggests that this mode of transmission is not a problem amongst dairy cows (Abb-Schwedler et al., 2014; Barto et al., 1982).

## MATERIALS AND METHODS

### Study area

The study was conducted from the middle of September 2016 to the middle of November 2016, in Laikipia County, approximately 200 km north of Nairobi. The county covers an area of 9462 km<sup>2</sup> and has an annual rainfall varying between 400mm to 750mm (The County Government of Laikipia, 2015). Two ranches in the area were included in the study: Mpala about 170 km north of Nairobi, situated almost in the middle of Laikipia, and Loisaba, situated about 40km north of Mpala (Figure 4). The two ranches were chosen due to convenience, availability and to limit the cost of travel.

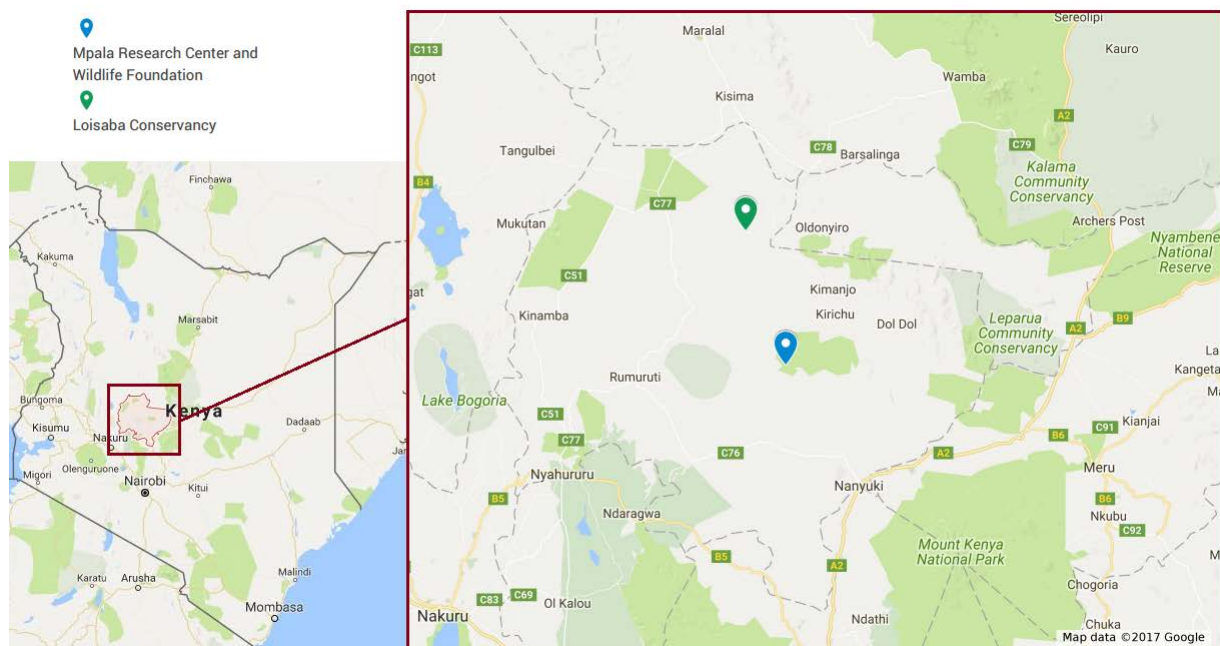


Figure 4. *The geographical location of Laikipia county and the location of the two ranches included in this study.*

### Study population

This observational study included 35 camels from the 2 different herds in the Laikipia area, 18 from Mpala Research Center and 17 from Loisaba Conservancy. One of the ranches was visited twice and the other was visited three times. During the visits to the herds, all the lactating females were sampled and milk samples from all lactating quarters were collected. This resulted in 13 individuals sampled once, 13 individuals sampled twice with 2 weeks in between, and 9 individuals sampled thrice at 2 weekly intervals. From the 35 camels, 261 quarter milk samples in total were collected.

### Mpala

The ranch is situated about 175 km north of Nairobi. The camel herd at Mpala consisted of 90 females of which 16 were lactating during the first and second visit and 10 during the third. One bull was kept with the herd for breeding purposes and the rest of the adult males were sold for meat. The camels are kept and managed in a semi-pastoralist way. Teat tying was not practiced. This ranch was visited 3 times at intervals of 2 weeks.

The herdsmen did not wash their hands before starting the milking or between animals. No cleaning of the udders was practiced. The milking was done completely by hand, milk-letdown was initiated by allowing the calf to suckle and the milking was done by two persons – one for each side.

### ***Loisaba***

The ranch is situated on a plateau to the northwest of Mpala research center, about 290 km north of Nairobi.

The camel herd at Loisaba consisted of 60 females of which 12 were lactating during the two visits. One bull was kept with the herd for breeding purposes and the rest of the adult males were kept in a separate herd for riding or sold for meat. The camels were kept and managed in a semi-pastoralist way. Teat tying was not practiced. This ranch was visited twice at 2-weekly intervals.

The herdsmen washed their hands before starting the milking but not between animals. No cleaning of the udders was practiced. The milking was done completely by hand, milk-letdown initiated by allowing the calf to suckle and the milking was done by three persons – one for each side and a third holding the milk-bucket.

## **Sample collection**

### ***Basic information including teat tying***

For each camel included in the study some basic information about age, days in milk, parity and previous cases of CM was collected by asking the herdsmen questions according to a pre-formulated questionnaire. The herdsmen were specifically asked about the use of teat tying, and the udders were visually inspected for signs of teat tying. During the collection of milk-samples, the udders were visually inspected for the presence of tick infestation.

### ***CMT***

The milk samples for the CMT were collected in such a way as to cause the least disruption to the milking process. The calves were allowed to suck their mothers to initiate milk letdown, and the milking commenced as per the usual routine in the herd. The first few streams of milk were discarded in order to reduce the amount of contamination from bacteria on the teat ends and from the teat canal. After that the milkers were instructed to squeeze a couple of streams of milk into the four wells on the paddle used for the CMT (Figure 5). The paddle was held with the handle facing forward, and milk from each quarter was squeezed into a separate well i.e. milk from each quarter was collected separately. The paddle was then slowly tilted to remove excess milk until an equal amount of milk remained in each well. A corresponding amount of readymade CMT-fluid (of the brand Bohvivet) was added to the well. The paddle was then rotated for 30 s and the reaction was scored 1-5 according to the Scandinavian system (Table 1). The CMT score was recorded on paper in combination with the animal's identity and later converted to a digital format. The CMT testing was done during the morning milking, and the milk samples for bacteriological analysis were collected at the following morning milking, since it was not possible for a single person to do both during the same milking.





Figure 5. The CMT-paddle used for testing the SCC of milk. (Daniel Goncalves 2016).

### **Milk samples**

The milk samples were collected in a similar way as for the samples for CMT in that milking was initiated by the calf. The first few streams of milk were discharged and then the milkers were instructed to squeeze one stream of milk into the test tube without touching the tube or the stream with their hands. No cleaning or disinfection of the udder was used apart from the flushing from the milk streams, to avoid interrupting milk let-down. The test tube was held at an angle of approximately 45 degrees (Figure 6). The collected samples were kept in a cooler with ice-packs and were frozen within 1-2 hours; they were kept frozen until delivered to the laboratory.



Figure 6. Collection of milk samples at Loisaba Conservancy (Set Bornstein 2016).

## Teat morphology

The morphology of the teats was recorded and measurements carried out digitally; the method was adapted from the one used by Ayadi et al. (2016). Two pictures of the left and right side of the udder were taken with a digital camera (Sony Alpha 5000) at a distance of approximately 50cm. A ruler was held below and in line with the teats, and was later used as a scale when analyzing the pictures. The pictures were then transferred to a computer, and using the program Image J, the following measurements of the teats were collected for each quarter; teat length (TL), and teat width (TW) (Figure 7). In the Mpala herd, pictures of the camels' individual brand were taken immediately after the pictures of the udder in order to later identify the individual to which the pictures of the udder belonged. In the Loisaba herd, the camels had no individual branding so the system was modified. The herdsman were asked for the camel's name, which was written down on paper and then a picture of the written name was taken immediately after the pictures of the udder.



Figure 7. Visual representation of the different teat measurements included in this study. In the bottom of the picture the ruler used for scale can be seen. Red line = teat length, blue line = teat width (Daniel Goncalves 2016).

## Bacteriological analysis

The milk samples were cultured by a commercial laboratory in Nairobi (Analabs laboratories ltd). Approximately 10µL milk were placed on an ovine blood agar plate using a reusable metal loop that was sterilized in a Bunsen burner flame between samples. The plates were then incubated at 37°C for 24-48h. An initial species differentiation was made based on a combination of visual examination of morphology (size, shape and color) and Gram-staining. The colonies on each agar plate were classified as *Streptococcus* spp, *Staphylococcus* spp, beta hemolytic staphylococci, Gram-negative rods or *Bacillus* spp. Growth of  $\leq 3$  bacterial colonies was considered as negative, unless the bacteria were determined to be beta hemolytic staphylococci. Samples with bacterial monoculture (growth of only a single bacterial species) were considered to come from quarters with IMI, samples with 2 different bacteria was defined as mixed growth and samples with growth of  $\geq 3$  defined as contaminated.

From all the samples with growth of bacterial monoculture, colonies were transferred to an ovine blood agar plates according to morphology and incubated for another 24-48h. If the growth on the new plate was in monoculture during visual examination, bacterial material was then collected using a metal loop and frozen in Tryptone Soy Broth with 10% glycerol and stored in Eppendorf tubes at -20°C until transport to Sweden. If the growth was not homogeneous, bacterial colonies of the “correct morphology” (i.e. the same morphology as the colonies from the original sample) were transferred to a new plate and incubated for another 24-48h. This process was repeated until homogeneous growth was found by visual examination, and then material was collected and frozen as described above.

### Storage during transport

The isolates were transported to Sweden using three different methods of storage during the transport, to determine which method should be used during transport in future studies. The methods used are described in table 5.

Table 5. A summary of the three different methods used in the storage of bacterial isolates during transport from the laboratory in Nairobi to the university in Sweden.

Method no.	Description
1.	Bacterial isolate transported frozen in Eppendorf tubes in Tryptone Soy Broth with 10% glycerol.
2.	Bacterial isolate frozen in Tryptone Soy Broth with 10% glycerol was regrown on ovine blood agar and bacterial material collected using a swab. This was done at the lab in Nairobi. The swab was then transported in a culturette with Amies medium and charcoal (Transsystem <sup>TM</sup> manufactured by Copan Italia).
3.	Bacterial material was collected from the agar with pure culture, using a swab with Aimes medium. This was done at the lab in Nairobi. The swab was then transported in a culturette with Amies medium and charcoal (Transsystem <sup>TM</sup> manufactured by Copan Italia).

The isolates were transported in multiple copies using both transport method 1 and 3 or method 2 and 3 (table 9). All isolates were transported in a cooler with icepacks. In Sweden the isolates were regrown on blood agar and the species identification was made using Matrix assisted laser desorption/ionization time of flight (MALDI-ToF) at the Swedish university of Agricultural Sciences (SLU) according to accredited methods. The values recommended by the manufacturer were used as cut-offs for identification ( $\geq 2.0$  for species-level identification, 1.7 to 1.9 for genus level identification and  $<1.7$  no reliable identification). The prevalence of bacterial contamination (defined as an isolate containing  $\geq 2$  species of bacteria) for the isolates was recorded.

## Statistical analysis

Descriptive statistics were used to present the data for TL, TW, age, parity and days in milk (DIM). The prevalence of IMI and SCM is presented using **point prevalence** ie. proportion of udder quarters with SCM/IMI during the first sampling for each individual quarter and as **period prevalence** ie. the proportion of udder quarters that developed SCM/IMI sometime during the whole study period. In addition to this the **incidence** of new cases of IMI or SCM during the study period was calculated as well. When comparing prevalence's with other studies it is **point prevalence** that is discussed unless anything else is specifically stated.

The median was used as cut-off in order to divide age, parity and days in milk into two categories that could be compared for differences in the prevalence of IMI or SCM. No categorization was used for TL and TW as they were normalized variables. In this study SCM was defined as CMT  $\geq 3$  and IMI as bacterial growth of monoculture

The Chi<sup>2</sup> test was used to compare differences in prevalence of IMI and SCM between the two groups mentioned above for age, parity, DIM. The Chi<sup>2</sup> test was also used to analyze differences between the three transportation methods in prevalence of bacterial contamination (ie  $\geq 2$  species) in the isolates. *P*- values  $\leq 0.05$  were considered as significant.

T-test and univariable mixed-effect linear regression analyses were used to investigate associations between TL or TW and age, parity, DIM, prevalence of SCM and IMI. Only data from the first sampling per camel were used in the regression analyses. A mixed-effect model was used in order to take into account the repeated observations within camel, i.e. that teats within a camel are more similar compared to teats between camels, and an identity covariance structure was used. *P*- values  $\leq 0.05$  were considered as significant.

The data were summarized and categorized using Microsoft Excel while the descriptive statistics and the Chi<sup>2</sup> test were done in Minitab 17, and the t-tests and the regression analyses were made in Stata 13.1.

## RESULTS

In total, 138 individual udder quarters from 35 camels were included in the study. Out of the 35 camels, 13 were sampled once, 13 were sampled twice, and 9 were sampled three times. One of the camels included in the study had 2 blind teats and one sample was lost during transport to the lab. In total, this resulted in 261 quarter milk samples.

Teat tying was not practiced in either herd according to the herdsmen, and no cases of teat tying was observed. No tick infestations of the udder were observed.

### Age, parity and DIM

The median age of the participating camels was 15 years, the median parity was three and the median DIM was 120. These data are summarized in Table 6.

Table 6. *Summary of age and lactation-related data of clinically healthy lactating camels (n=35) in two stationary camel herds in Kenya. The information on results of the California mastitis test (CMT) is presented at udder quarter level.*

	Median	Min	1 <sup>st</sup> quartile	3 <sup>rd</sup> quartile	Max
Age (years)	15	13	14	17	17
Parity	3	1	2	4	4
Days in milk	90	7	90	180	360
CMT <sub>1</sub>	1	1	1	2	4
CMT <sub>2</sub>	1	2	1	2	4

1 = All sampled quarters, n = 253, 2 = Only first sample for each quarter n= 134

### CMT and prevalence of SCM

From 34 camels, 253 quarter samples were analyzed using the CMT. The majority (88%) of udder quarters had a CMT score of 1 or 2 and no udder had more than 2 quarters with SCM (Figure 8).

The median CMT-score was 1 when considering all sampled quarters, or 2 when taking into account only the first sampling of each individual (Table 6).

The point prevalence for SCM was 11% (15/134) at udder quarter level. The incidence of new inflammations during the study period (8 weeks) was 8%. The period prevalence for SCM was 19% (25/134) at udder quarter level.

The point prevalence of SCM on camel level was 29%, (10/34) camels had a CMT of  $\geq 3$  in one or more udder quarter at the first sampling occasion. The incidence of new inflammations during the study period was 43%. The period prevalence was 46% (16/34) at camel level.



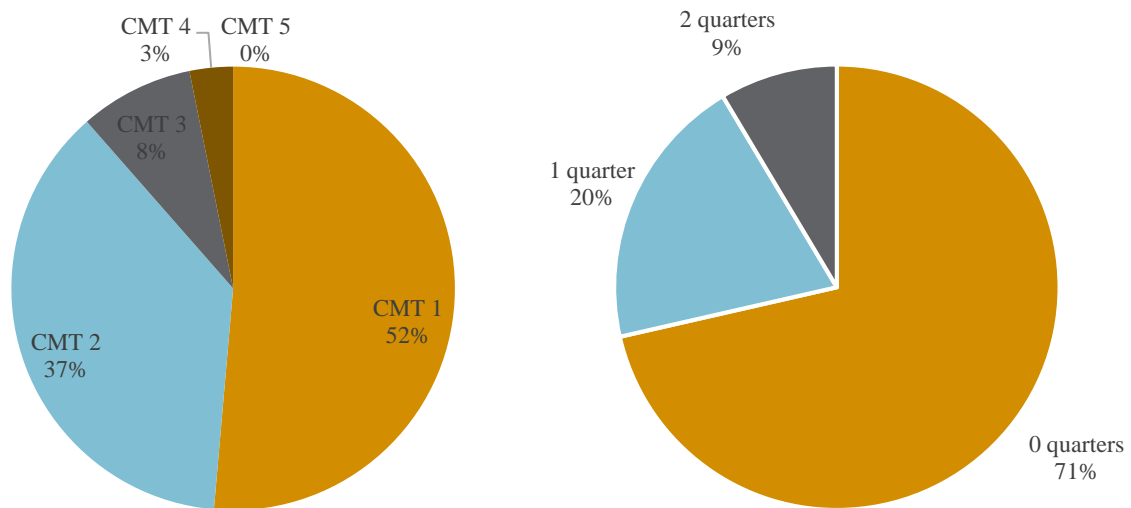


Figure 8. Left: *The distribution of CMT-scores in udder quarters from camels with clinically healthy udders (n=253).* Right: *The distribution of camels with SCM in one or more quarters (n=34).*

### Prevalence of IMI

In the 261 milk samples, bacterial growth of one or more species was found in 83% of the samples. The prevalence of IMI was 48% (table 7).

When taking in account only the first sample from each individual camel, the point prevalence of IMI was 43% (table 7). During the study period (8 weeks) the incidence of new infections was 33%. The period prevalence of IMI for the included quarters was 61% (85/140).

Table 7. *Comparison of the bacteriological results for all quarter milk samples included in the study (total) compared to the bacteriological results for quarter milk samples from the first sampling occasion for each camel (point)*

	Prevalence	
	Total	Point
N	261	138
IMI (monoculture)	124 (48%)	59 (43%)
Mixed growth (2 species)	92 (35%)	59 (43%)
Contamination ( $\geq 3$ species)	1 (0%)	1 (1%)
No growth	45 (17%)	20 (14%)

The point prevalence of IMI was 89% (31/35) on camel level during the first sampling occasion. The incidence of new infection during the study period was 25% on camel level. The period prevalence was 91% (32/35) on camel level.

### **Bacterial species present in IMI**

Originally bacteriological isolates from all samples with IMI were planned to be transported to Sweden for species identification. However, due to difficulties during the lab work, only isolates from 43 out of the 124 samples with IMI were done in time and able to be transported to Sweden. Some isolates were transported in multiple copies using both transport method 1 and 3 or method 2 and 3 (table 9).

Among the 43 isolates from quarters with IMI the about 47% of the isolates were *Staphylococcus* spp. and 28% *Streptococcus* spp. *Staphylococcus simulans* (n=11) was most commonly identified followed by *Str. pluranimalium* (n=7) (Figure 9).

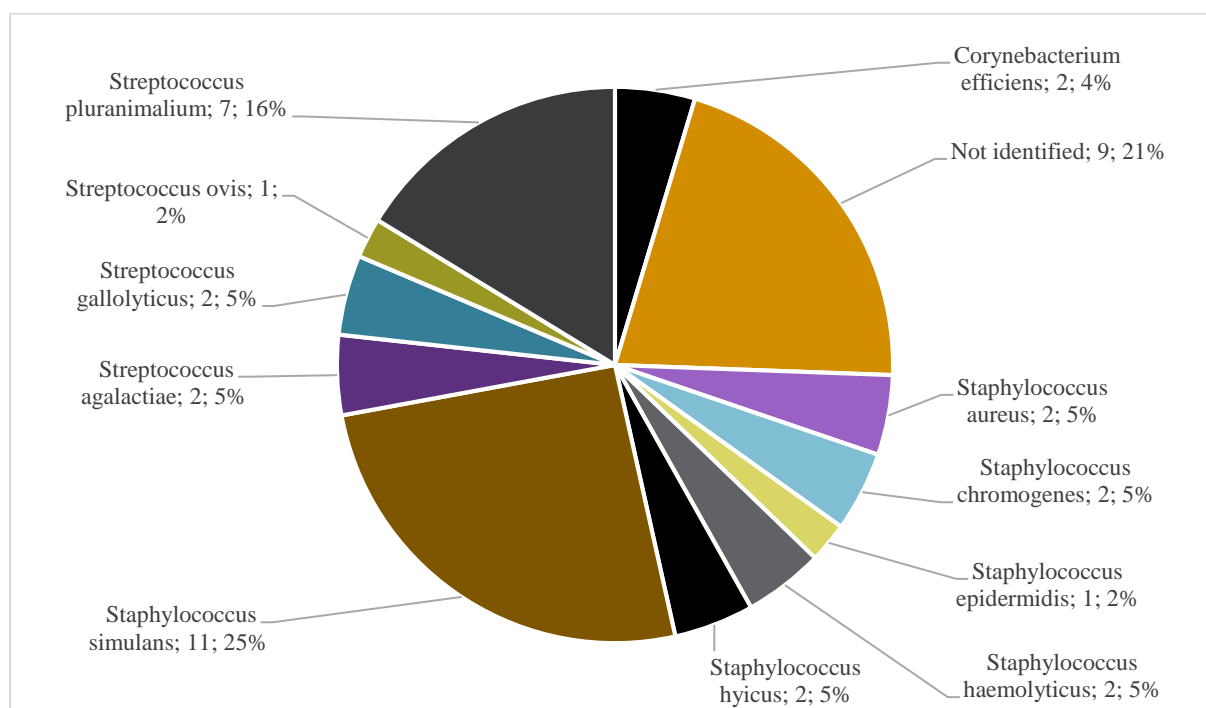


Figure 9. The distribution of bacterial species identified using MALDI-TOF on bacterial isolates from quarter milk samples (n=43) from camels with IMI.

Among the 8 isolates from udder quarters with **both** SCM and IMI, *Staphylococcus simulans* (2/8), *Streptococcus pluranimalium* (1/8), *Staphylococcus aureus* (1/8), *Corynebacterium efficiens* (1/8) was identified and 3/8 isolates was not identified.

### ***Differences in contamination during transport between different methods of transport***

After processing at the lab in Nairobi, pure isolates of all the bacterial species found in the milk samples were transported to Sweden. However, when analyzed at SLU 39% of the isolates contained at least two bacterial species. The number of “contaminated” isolates differed depending on the method of storage chosen for transport to Sweden (Table 8).

Table 8. *Summary of the number of isolate samples that contained  $\geq 2$  species, and a comparison between three different methods of transport of bacteriological isolates from the laboratory in Nairobi, Kenya to the University in Uppsala, Sweden*

Method no.	Description	No. of contaminated isolates (%)
1.	Transported frozen in Eppendorf tubes in Tryptone Soy Broth with 10% glycerol.	22/25 (88)
2.	Isolate frozen in Tryptone Soy Broth with 10% glycerol was regrown on ovine blood agar and collected using a swab. The swab was then transported in a culturette with Amies medium and charcoal (Transsystem <sup>TM</sup> manufactured by Copan Italia).	17/40 (43)
3.	isolate collected from the agar immediately before freezing using a swab. with Aimes medium. The swab was then transported in a culturette with Amies medium and charcoal (Transsystem <sup>TM</sup> manufactured by Copan Italia).	9/37 (24)



## Teat morphology

The average TL was 51 mm and the average TW was 32mm (table 9). Differences in TL between front and hind teats were observed. The right front teat (RF) was significantly longer than both the right hind teat (RH) ( $P=0.004$ ) and the left hind teat (LH) ( $P<0.001$ ). The left front teat (LF) was significantly longer than the LH teat ( $P=0.008$ ). There was no significant difference in length between RF and LF teats or between RH and LH teats. For a summary of the measurements for TL see Figure 10.

Table 9. The mean and standard deviation (StDev) for teat length (TL) and teat width (TW) for all udder quarters measured in the study.

	N	Mean (mm)	StDev (mm)
TL	123	51	15
TW	123	32	8

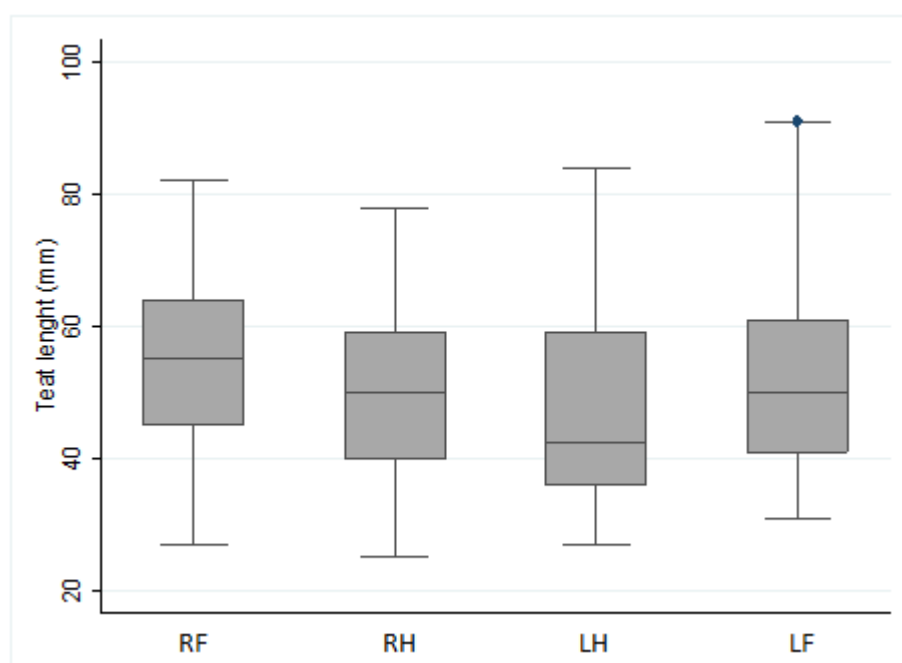


Figure 10. Box and whisker plot over the length of all four teats in 35 camels with clinically healthy udders. The box shows the lower quartile, median and the upper quartile, and the whiskers shows the upper and lower adjacent values (1.5 inter-quartile range from the lower and upper quartile, respectively). The dots are outliers. RF=right front teat, RH=right hind teat, LH=left hind teat, LF=left front teat.

A difference in TW was observed between front and hind teats. The RF teat was wider than the RH teat ( $P<0.001$ ) and LH teat ( $P<0.001$ ). The LF teat was wider than the LH ( $P=0.002$ ) and the RH teat ( $P=0.001$ ). No significant difference was observed between the RF teat and the LF teat or between the RH and the LH teat. For a summary of the measurements for teat width see Figure 11. A significant association between TL and TW was found ( $r=0.77$ ,  $p<0.00001$ ), see figure 12 for details.

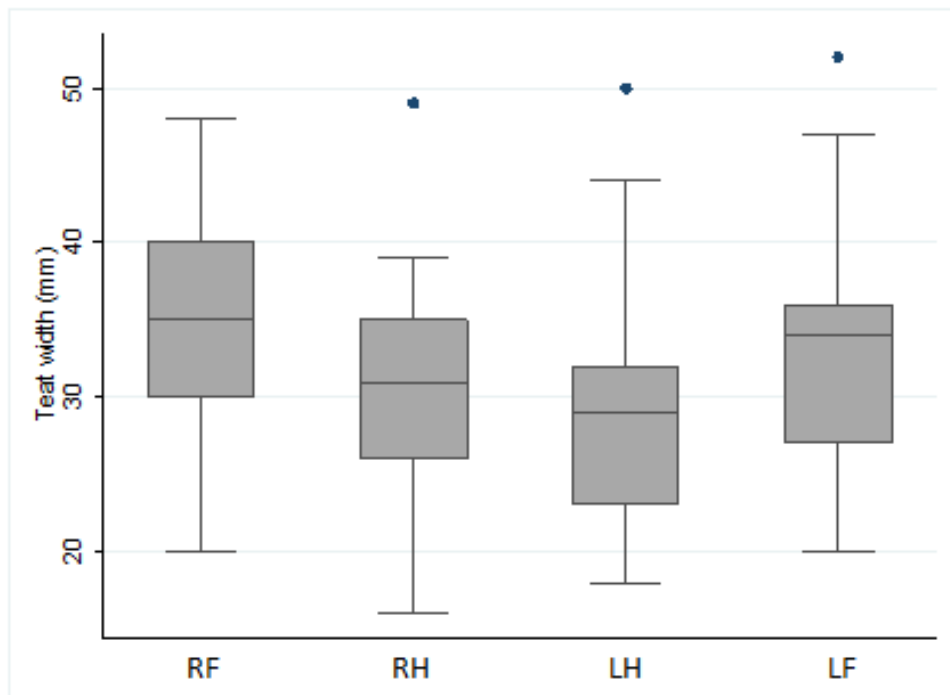


Figure 11. Box and whisker plot of the width of all four teats in 35 camels with clinically healthy udders. The box shows the lower quartile, median and the upper quartile, and the whiskers shows the upper and lower adjacent values (1.5 inter-quartile range from the lower and upper quartile, respectively). The dots are outliers. RF=right front teat, RH=right hind teat, LH=left hind teat, LF=left front teat.

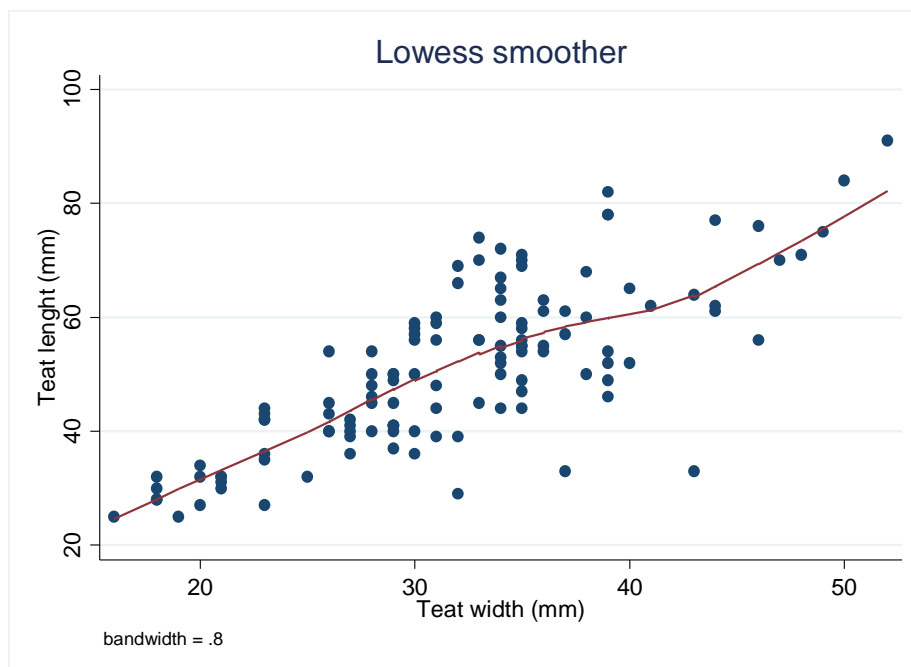


Figure 12. Scatter plot of the significant association between teat length and teat width from 140 teats in 35 camels with clinically healthy udders.

### Associations between teat morphology and camel factors and udder health

According to the results of the univariable mixed-effect regression model, camels in their 1<sup>st</sup> parity had wider teats (approximately 16 mm wider) than camels in their 4<sup>th</sup> parity ( $P=0.005$ ). Camels in their 2<sup>nd</sup> parity had wider teats (approximately 8 mm wider) than camels in their 4<sup>th</sup> parity ( $P=0.01$ ). For a summary of the TL and TW for each parity see table 10.

Table 10. An overview of the mean and standard deviation (StDev) for teat length (TL) and teat width (TW) for all udder quarters measured in the study, grouped by parity and the total for all udderquarters for comparison.

Parity	N	TL mean (mm)	StDev (mm)	TW mean (mm)	StDev (mm)
1	4	68	8	43	5
2	24	55	12	35	8
3	39	32	12	32	5
4	28	43	12	27	8
Total	123	51	15	32	8

No **significant** difference in TL or TW was found between camels in their 3<sup>rd</sup> and 4<sup>th</sup> parity. No **other** significant associations were found between TL or TW and age, DIM, SCM or IMI.

## **DISCUSSION**

### **Prevalence of SCM**

In this study, the point prevalence of SCM was 9% of the udder quarters. This is lower than reported in a number of studies conducted on camels in Ethiopia and also lower than reported from camels in Pakistan. The animal-level prevalence of SCM was 29% which is at the lower end of the range (25 – 67%) reported in the literature (Ahmad et al., 2011; Husein et al., 2013; Mohammed et al., 2005; Obied et al., 1996; Regassa et al., 2013; Seifu and Tafesse, 2010). The low prevalence of SCM when compared to other studies is most likely because CMT  $\geq 3$  was chosen as the definition of SCM in the present study compared to CMT  $\geq 2$  used in some other studies that reported higher prevalence (Husein et al., 2013; Regassa et al., 2013; Ahmad et al., 2011). Another factor that might contribute to a lower prevalence of SCM is that the present study investigated camels kept under a semi-pastoralist management, compared to Husein et al. (2013), Regassa et al. (2013) and Ahmad et al. (2011) who investigated camels kept by true pastoralists. This means better access to clean water which might mean better milking hygiene, better access to antibiotic treatment for cases of CM (and other diseases) and regular antiparasitic treatments. Ahmad et al. (2011) noted heavy tick infestations on the udders of their camels. The camels included in this study had no ticks due to regular treatments with anti-parasitic agents.

Another important difference compared to the other studies is that teat tying was not performed in the herds included in the present study, whereas this practice, which predisposes to mastitis, was used by almost all herds included in the other studies mentioned above (Ahmad et al., 2011; Husein et al., 2013; Mohammed et al., 2005; Obied et al., 1996; Regassa et al., 2013; Seifu and Tafesse, 2010).

### **Prevalence of IMI**

In the present study the quarter level point prevalence of IMI was 43% which is comparable to the 44% to 67% reported in the literature (Abdurahman et al., 1995; Obied et al., 1996; Matofari et al., 2005). It is on the lower end of the reported range which might be expected since the camels in the present study were kept under semi-pastoralist management without harmful practices such as teat tying and with access to antibiotic treatments and regular anti-parasitic treatments (discussed in the section about prevalence of SCM).

The point prevalence of mixed bacterial growth was 43% which seems high, considering that the rates reported in the literature are 4% to 8% (Abdurahman et al., 1995; Obied et al., 1996). Cleaning or disinfection of the teats could have been implemented in order to reduce the bacterial contamination during sampling. Some authors have used cotton balls dipped in 70% ethanol after the pre-milking stimulation by the calves (Ahmad et al., 2011; Husein et al., 2013; Regassa et al., 2013). However, this would mean a larger disturbance in the milking process and probably require more manpower. Another method of disinfection would be to spray the teats with alcohol using a spray bottle. However, this would require some drying to remove excess alcohol, and mean a larger disturbance in the milking process. When designing the study the use of disinfection was discussed and ultimately decided against, due to the above mentioned problems and the experience of one of the local supervisors that clean samples could

be obtained from camels even without disinfection. There were also some concerns about the safety for the person doing the disinfection, since the camels were not used to any kind of cleaning of the udders. It has been reported that camels react negatively to the feeling of ethanol on the skin of the teats (Younan et al., 2001). Looking at the high prevalence of mixed growth, it would probably have been wise to include disinfection.

### **Bacterial species present in IMI**

The most commonly identified bacterium in 43 udder quarters with IMI was *S. simulans* (26%). Other CNS-species found were *S. hyicus* (5%), *S. haemolyticus* (5%), *S. chromogenes* (5%) and *S. epidermidis* (2%). In total, the combined prevalence of CNS in udder quarters with IMI was 43% in the present study. This contrasts with the findings of Obied et al. (1996) who reported a prevalence of 13% CNS in camel udder quarters with IMI.

The prevalence of CNS in udder quarters with IMI **and** SCM was 29%. This is in line with the 15 – 38% prevalence of CNS in udder quarters IMI **and** SCM amongst camels reported in the literature (Ahmad et al., 2011; Regassa et al., 2013; Seifu and Tafesse, 2010). However, the prevalence for bacterial species amongst udder quarters with IMI **and** SCM reported in the present study should be interpreted with caution due to the low numbers of udder quarters with IMI **and** SCM included (n=8).

Many species from the CNS group can be found on the skin and teats (Piessens et al., 2011); since the present study did not use any disinfection of the teats, it is possible that some of the CNS found were originally from the skin of the teat. However, the fact that the CNS-species identified were the same CNS-species that most commonly cause SCM in dairy cattle (Piessens et al., 2011; Pyorala and Taponen, 2009; Thorberg et al., 2009) suggests that this might not be the case. That the bacteria grew in monoculture and that the overall prevalence was in line with some of the earlier studies gives further evidence to this speculation.

Regarding the individual CNS species Obied et al. (1996) found 13% prevalence of *S. epidermidis* in quarter milk samples with IMI; the present study found 2% prevalence in quarter milk samples with IMI. Other CNS species identified in quarter milk samples with IMI in the present study are *S. simulans* was identified in (26%), *S. chromogenes* (5%) and *S. haemolyticus* (5%), which all have been identified in udder quarters from camels with CM before (Alqurashi et al., 2013). The prevalence of *S. simulans* in the present study is higher than reported by Alqurashi et al. (2013) while the prevalence of *S. chromogenes* and *S. haemolyticus* is approximately the same. These differences are not unexpected since considerable variability in the prevalence of individual CNS-species between herds has been found in cattle (Piessens et al., 2011). A similar variability probably exists in camels, especially when comparing studies from different countries and seasons.

Regassa et al. (2013) found that the prevalence of *S. intermedius* was 5% and the prevalence of *S. hyicus* was 11% in quarter milk samples with IMI **and** SCM. While Mohamed et al. (2016) found 1% prevalence of *S. hyicus* in udder quarters with IMI **and** SCM. The only CNS species found by the present study in udder quarters with IMI **and** SCM were *S. simulans* (29%), but this result should be interpreted with caution as mentioned before.

The second most common bacteria identified was *Str. pluranimalium* (17%). To the knowledge of the author this bacterium has not been isolated in milk from camels before. The bacteria has been found in the genital tract and tonsils in cattle, in the tonsils of goats and has been reported to cause empyema in humans (Aryasinghe et al., 2014; Devriese et al., 1999). Like other streptococci, *Str. pluranimalium* can be found as a part of the normal flora and since the present study did not use any disinfection of the teats, the findings of *Str. pluranimalium* could be either contamination from the normal flora of the skin or an opportunistic infection of the mammary gland (Guccione et al., 2016). It is likely that this finding represents an infection of the mammary gland since the bacteria was found in monoculture and has been isolated in mastitis cases in cattle and in SCM in buffaloes before (Devriese et al., 1999; Guccione et al., 2016).

Another species whose prevalence differs from other studies is *S. aureus*. In this study the IMI prevalence was 5% while Obied et al. (1996) reported 17% prevalence in quarter milk samples from camels with IMI. The low prevalence of IMI with *S. aureus* in the present study is surprising considering that IMI with *S. aureus* has been reported to be common among camels in Kenya (Younan et al., 2001).

In udder quarters with IMI **and** SCM Seifu and Tafesse (2010) who reported a prevalence of 3% for *S. aureus* compared to the 14% in the present study (figure 9). The difference in prevalence could probably be due to the low number of samples with IMI **and** SCM included in the present study.

Two factors that might influence the prevalence of different species of bacteria are climate and season, thus making it harder to compare between different studies. In Kenya there is a big increase in environmental contamination of the udder during the wet season compared to the dry season in which the present study was performed, and a similar dynamic is expected to be present in other East African countries as well.

### **Bacteriological sources of errors**

A methodological source of error included in this study is that the subset quarter milk samples with IMI analyzed by MALDI-ToF was not chosen in a random way. Originally it was planned that all isolates identified in samples with IMI would be analyzed by MALDI-ToF. However, the lab was not able to do the initial growth of all quarter milk samples in time for them to be transported to Sweden for analysis by MALDI-ToF. Only 58 quarter milk samples were ready in time; of these IMI was identified in 42 samples. All quarter milk samples collected were mixed together during the transport to the lab, and the lab workers were told to do the samples in any order. This means that the selection of samples can be considered to be at least pseudo-random. The selection was made without any interference from the author and is at least free from any intentional bias.

Contamination during the collection of milk samples due to the lack of disinfection has already been discussed.

Problems with contamination in the laboratory or during transport to Sweden must also be considered when interpreting the bacteriological results. After transport to Sweden, many of the supposed/reported isolates contained more than one species of bacteria, i.e. an isolate that

was supposed to contain one species of staphylococci contained an additional species as well. Three possible reasons for this were identified:

1. Contamination of the isolates during lab work
2. Re-used old Eppendorf tubes were used for storage of the frozen isolates
3. More than one isolate was taken from the plate.

Contamination during the lab work is possible. This contamination would have to happen after the lab workers had identified the number of different bacterial species grown from one quarter milk sample, either during the process of creating the bacterial isolates, or during the process of collecting bacterial isolates for freezing. Any contamination occurring before the initial inspection and species identification (i.e. from the time that the milk sample was placed on the agar to the time that it was inspected again) would appear as an increase in the mixed growths reported from the lab.

Looking at the lab environment, the possible sources of contamination include flies in the lab, lab workers using their mobile phones during the processing of samples, mistakes in the sterilization of the reusable metal loop used for streaking of samples, and contamination during storage in the fridge.

Another possibility that might explain why the frozen isolates fared worse than the swabs, is that the lab re-used old Eppendorf tubes for storage of the frozen isolates. These tubes had been used before and were sterilized by boiling and bathing in iodine, but these tubes are made of plastic and are not meant to be re-sterilized. There could be a risk that these tubes were not properly sterilized, and bacteria could remain and contaminate the “new” samples, or the tubes might become warped during the boiling and therefore not close properly, allowing entry of contaminating bacteria either during the storage in the freezer or during transport. The fact that the lab was using old tubes was not known beforehand, and was only discovered during the last week of the fieldwork.

Another possibility is that the lab workers mistakenly identified mixed growth as pure cultures. Since the initial identification and categorizing of the initial growth from the quarter milk samples relied on visual inspection of the morphology of the bacteria, the lab workers’ skill in identifying different bacteria might also have an impact. Mastitis bacteriology is not the usual focus of the lab even if they have previously done some work in the field.

The time spent in transport was 3 days i.e. between leaving the lab and regrowth in Sweden. If the isolates were 100% pure, the time spent in transport should not matter; however the time spent in transport might increase the effect of a possible contamination. The effect of the long transport should be mitigated by the fact that the isolates were kept cold during the whole transport and no leakage during transport was identified.

We were not able to identify 21% of the bacterial species found in quarters with IMI using MALDI-ToF. Most of the isolates that were not identified had scores in the range of 1.3 – 1.6, which is far from the manufacturers recommended cut of values ( $\geq 2.0$  for species-level

identification, 1.7 to 1.9 for genus level identification and  $<1.7$  no reliable identification). This could be because the strains of the bacteria encountered in udder quarters with IMI amongst camels are not included in the database used for identification. This problem have been reported to occur in the past, and it is possible that adding the specific bacterial strains found in IMI amongst camels to the database are not first on the priority list (Carbonnelle et al., 2012).

Another possibility is that the bacteria we tried to identify were not the bacteria from the isolate but rather the contaminating bacteria. Since a high number of the isolates were ciontanimated we can not be sure thaty

### **Correlation between SCM and IMI**

In the present study, IMI was identified in 59% of milk samples from udder quarters with SCM. In comparison, Regassa et al. (2013) identified IMI in 78% of udder quarters with SCM, Ahmad et al. (2011) in 91% of udder quarters with SCM and Seifu and Tafesse (2010) in 83% of udder quarters with SCM.

A high rate of bacterial contamination during sampling might have hidden the true prevalence of IMI, and this might explain the lack of a significant association between SCM and IMI. Bacterial growth of one or more species (i.e. both mixed growth and IMI) was found in 93% of the samples from udder quarters with SCM, and using this material the association between SCM and IMI tended to be significant, which gives further support to this hypothesis.

### **Correlation between SCM and camel factors, IMI and camel factors**

Significant associations were not found between any of the camel factors (age, parity and DIM), and SCM or IMI. In previous studies, significant associations between these factors and SCM or IMI were found in camels (Abera et al., 2010b; Ahmad et al., 2011; Regassa et al., 2013) as well as in cattle and other species (Abebe et al., 2016; East et al., 1987). Possible reasons for the different result in the present study are the small sample size and the fact that no record keeping was practiced in the herds included in the study. All data about age, DIM and parities comes from the memory of the herdsman.

### **Teat Morphology**

The average TL and TW found in this study were smaller than those of lactating camels reported in Saudi Arabia (Ayadi et al., 2016). This difference is most likely not due to a true difference in methodology since the methods used in this study are based on the ones used by Ayadi et al., (2016). One explanation for the difference in teat size is that there has not been any selection for teat shape in Kenyan camels. The Saudi camels are milked by machine and this might have created a tendency to select animals with longer and wider teats. Such teats fit better in the liners/teat cups of the milking machine, enabling more efficient milking and a higher milk yield. Another thing to consider is that considerable variability in TL between different breeds of camels have been reported, and the present study did not look at the race of the camels at all (Abdallah and Faye, 2012).

Significant differences in TL and TW were found between front and hind teats, but not between right side and left side teats. The present study found that the front teats were wider and longer



compared to the rear teats which is in line with what Ayadi et al. (2016) found among lactating dairy camels in Saudi Arabia. Wider and longer front teats compared to hind teats have been reported for dairy cattle as well (Rønningen and Reitan, 1990; Zwertvaegher et al., 2012)

Camels in their 1<sup>st</sup> and in their 2<sup>nd</sup> parity had significantly wider teats than camels in their 4<sup>th</sup> parity. However, these results should be interpreted with caution due to the low sample size (N=26) and the fact that the number of parities reported in this study comes from the memory of the herdsmen since no record keeping were practiced in the herds included in the study. In cattle the opposite correlation have been reported, i.e. increased TW with an increased number of parities (Zwertvaegher et al., 2012).

No significant association between TL or TW and SCM or IMI was found. The high incidence of suspected bacterial contamination during sampling might have distorted the data and hidden possible associations.

## CONCLUSIONS

According to our definition of SCM the quarter-level point prevalence of SCM was 11%, the incidence of new inflammations during the study period (8 weeks) was 8% and the quarter level period prevalence of SCM was 19% in the two camel herds studied.

According to our definition of IMI the quarter-level point prevalence of IMI was 43%, the incidence of new infections during the study period (8 weeks) was 33% and the period prevalence was 61% in the two camel herds studied.

The bacterial species identified in quarter milk samples with IMI were: *S. simulans* (26%), *Str. pluranimalium* (17%), *S. hyicus* (5%), *S. haemolyticus* (5%), *S. chromogenes* (5%), *S. aureus* (5%), *S. agalactiae* (5%), *S. gallolyticus* (5%), *S. ovis* (2%), *C. efficiens* (2%) and *S. epidermidis* (2%). We were not able to identify 21% of the bacterial species found using MALDI-ToF.

The average TL was 51mm and the average TW was 32mm. There was a strong correlation between TL and TW. Front teats were significantly longer and wider than hind teats but no significant difference was found between left and right side for either front or hind teats.

The association between IMI and SCM was not significant. A significant association was found between TW and parity, where camels in parity one or two had wider teats than camels in parity four. No other significant associations were found for the variables included in the study.

While the results in this study should be interpreted with caution due to troubles during the bacterial analysis the high prevalence of IMI found still suggests that further studies to determine the true prevalence of IMI and SCM and intervention studies on milking hygiene or other ways to lower this prevalence might greatly benefit the pastoralists and camels in Kenya.

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